PLANT BREEDING USING NEXT GENERATION SEQUENCING

FIELD OF THE DISCLOSURE

[0001] The present disclosure pertains to novel methods for screening/detecting of plants, in particular by a multiplex PCR-based combined with a next-generation sequencing approach to analyze a plurality of characteristics (e.g. target sequences) of an individual plant in parallel.

BACKGROUND

[0002] Plant cultivars and varieties with yield, nutritional quality and agronomic performance optimized for different environments are needed to supply the growing global population (UN, 2008). Optimized characteristics can be achieved by conventional breeding, but this takes up to 8 years because phenotype-based testing requires fully grown plants (Borlaug, 1983, ISAAA, 2014). The speed and efficiency of plant breeding can be improved by adopting technologies such as reverse breeding, marker assisted selection (MAS) and genetic modification, all of which have advantages and disadvantages (He, Zhao, Laroche, Lu, Liu and Li, 2014, Jonas and de Koning, 2013, Nakaya and Isobe, 2012, Varshney, Nayak, May and Jackson, 2009). These modern techniques are steadily replacing or augmenting classical breeding approaches.

[0003] Barley for example is an important cereal crop which has been cultivated for thousands of years (Reets and Léon, 2004). It ranks fourth in terms of production volume behind maize, rice, and wheat, and is primarily used for food, feed and the production of alcoholic beverages (FAO, 2014). Barley is cultivated in different climates, soils and environments, and is exposed to diverse forms of abiotic and biotic stress.

[0004] The development of new sequencing instruments ("next generation" or "massively parallel") had a massive impact on genomics, since next generation sequencing (NGS) enables the generation of millions to hundreds of millions of reads in the same sequencing run. As a consequence, this technique has already found numerous applications in molecular and evolutionary biology, metagenomics, and clinical areas, such as in the analysis of genomes, in human genetics, forensics, prenatal screening, early detecting of cancer, etc.

[0005] Many NGS platforms differ in engineering configurations and sequencing chemistry. However, most sequencing approaches use an in vitro cloning step to amplify individual DNA molecules, because their molecular detection methods are not sensitive enough for single molecule sequencing. Thus, the recent sequencing platforms all share the technical paradigm of massive parallel sequencing via spatially separated, clonally amplified DNA templates or single DNA molecules in a flow cell. This design is very different from that of Sanger sequencing—also known as capillary sequencing or first-generation sequencing—which is based on electrophoretic separation of chain-termination products produced in individual sequencing reactions.

[0006] Next generation sequencing generates large amounts of data in a short time by producing thousands or even millions of reads in parallel. The increasing throughput and falling costs of NGS have encouraged multiple applications in different areas of the life sciences, including medicine (Metzker, 2010) and agriculture (Elshire, Glaubitz, Sun, Poland, Kawamoto, Buckler and Mitchell, 2011, Mascher, Wu, Amand, Stein and Poland, 2013, Teixeira, Fortes, Pinheiro and Pereira, 2014, You, Huo, Deal, Gu, Luo, McGuire, Dvorak and Anderson, 2011).

[0007] For example, the high-throughput sequencing of large numbers of amplicons has been used to genotype the human leukocyte antigen (HLA) locus (Bentley, Higuchi, Hoglund, Goodridge, Sayer, Trachtenberg and Erlich, 2009, Holcomb, Hoglund, Anderson, Blake, Bohme, Egholm, Ferriola, Gabriel, Gelber, Goodridge, Hawbecker, Klein, Ladner, Lind, Monos, Pando, Proll, Sayer, Schmitz-Agheguian, Simen, Thiele, Trachtenberg, Tyan, Wassmuth, White and Erlich, 2011) and to determine zygosity in transgenic maize (Fritsch, Fischer, Wambach, Dudek, Schillberg and Schroper, 2015).

[0008] EP 2 200 424 B1 discloses a method for the selection of a population of plants that have an artificial mutation in a desired genomic area that may lead to improved genetic variation and improved phenotypes. After cultivating the plants in a defined order, the genomic DNA is isolated, pooled and parts of the desired genomic area comprising the inserted mutation is amplified. After the amplification, the amplicons will be sequenced and compared with a reference sequence. The several plant pools may be identified with a barcode sequence. Furthermore, EP 1 929 039 B2 discloses a high-throughput screening method for the detection of specific mutations in a plant population using next generation sequencing methods.

[0009] However, it is an object of the present disclosure to provide novel and improved methods for screening and/or selecting plants.

SUMMARY OF THE DISCLOSURE

[0010] The present disclosure pertains to novel methods for selecting/detecting a plant from a plant population by genotyping, in particular for the use of plant breeding.

[0011] The present disclosure pertains to novel methods for plant selection useful for plant breeding processes, in particular by a multiplex PCR-based approach to analyze a plurality of characteristics (e.g. target sequences) of individual plants in parallel. First after the amplification, the amplification products (amplicons) of a plurality of plants are pooled and sequenced together by new sequencing instruments/techniques ("next generation" or "massively parallel"). Due to the use of barcode sequences a plurality of plants can be examined in parallel.

[0012] The methods according to the present disclosure are rapid and high-throughput analysis methods useful for plant breeders and farmers. These methods provide robust genotyping data that allow the rapid determination of genotype and zygosity. These methods can be used to genotype large panels of plants because up to 80 million individual reads can be produced in one sequencing run, and samples from different lines and/or traits can be pooled after the amplification step. These findings are significant because plant breeders may need to screen large populations for multiple traits in parallel. The methods provide further a simple and inexpensive approach for the rapid and accurate genotyping of natural polymorphisms e.g. in barley, which can also be applied in many other economically relevant crop species.

[0013] One advantage of the methods according to the present disclosure is that the amplification products could be allocated to an individual plant and to a reference sequence.